## REMARKS

The Applicants appreciate the Examiner's thorough examination of the subject application. Applicants request reconsideration of the subject application based on the following remarks.

Claims 8, 10-13, 15-18, 20-22 are pending, claims 9, 14, and 19 have been cancelled, and claims 8, 10-13, 15-18, 20-22 have been amended. Support for the amendments to claims 8, 13, and 18 can be found in claims 9, 14, and 19. Amendments to the dependent claims were made merely to remove claim dependency from now cancelled claims. No new matter has been added by virtue of these amendments. Support for such amendments can be found throughout the specification and in the original claims of the application.

Claims 8-18 were rejected under 35 U.S.C. §112, first paragraph, allegedly because the specification, while being enabling for the claimed methods to the extent of *in vitro* practice, wherein the germ cell is sperm and the high energy beam is near UV light (330-360 nm), does not reasonably provide enablement for all other methods embraced by the claims.

Claims 8-22 were rejected under 35 U.S.C. §112, second paragraph, as being allegedly indefinite for failing to particular point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 8, 13, and 18, as amended, comply with all the requirements of 35 U.S.C. §112, including the requirements of §112, first paragraph and §112, second paragraph.

Withdrawal of the §112, first paragraph, and §112, second paragraph, rejections is thus requested.

Claims 8-22 were rejected under 35 U.S.C §102 (b) as being allegedly anticipated by Chakrabarti (*Genetics*, 1983, 103:109-123), Grunwald #1 (*Genet. Res.*, 1991, 59:93-101),

Grunwald #2 (*Genet. Res.*, 1001, 59:103-116) taken with Thomas (*Mol. Cell. Biol.*, 1996 16(5):2537-2544).

The rejection is traversed.

The present invention provides an efficient method of mutating a gene of vertebrates and analyzing the mutated gene. Applicants have surprisingly discovered that psoralen derivatives causes a mutation in a vertebrate's genome upon exposure to UV-light. More particularly, Applicants have discovered that irradiating a mixture of DNA and a psoralen derivative with UV light induces a small deletion in the vertebrate's genome. Applicants have further succeeded in cloning the mutated gene by utilizing the small deletion as a marker (See page 15 line 6 to page 16 line 5 in the present specification).

In contrast, none of Chakrabarti et al., Grunwald et al. #1 or Grunwald et al. teach or suggest mutating a gene by incorporating a **small deletion in vertebrate's genome** or cloning such a mutated gene by the methods provided by the invention.

As the reference is understood, Chakrabarti et al. recites that "several observation reported here suggested that an appreciable fraction of γ–ray-induced zebrafish mutations are **long deficiencies**" (See page 120 line 6-8 in Chakrabarti et al., emphasis added). Thus, the long genome deficiencies induced by exposure to γ–ray irradiation taught by Chakrabarti make it difficult to isolate a mutation at a level of single gene locus.

Grunwald et al. #1 teaches mutation methods using UV light which induce **point mutations**, flameshift mutations, but rarely large deletions or mutations (See page 93, right column, line13-15).

Grunwald et al. #2 recites that "ENU-induced mutations are probably point mutations" (See page 115, paragraph(iv) ). Applicants note that cloning of point mutated genes typically requires laborious procedures such as genetic mapping and chromosomal walking.

In contrast, the present invention provides methods of gene mutation in which a small deletion in vertebrate genome is generated by irradiation of sperm cells contacted with a psoralen derivative with UV light. Psoralen derivatives are DNA cross-linking agents which induce small deletions in vertebrate genome by inducing nucleotide recombinational repair or nucleotide excision repair in the crosslinked interstrand site of DNA. The small deletion in vertebrate genome can be utilized as a marker for cloning the mutated gene.

As discussed in the specification, Applicants have successfully cloned the mutated gene (edw gene) by Representational Difference Analysis (RDA) in which the small deletion is utilized as a marker (See page 15 line 6 to page 16 line 5 in the present specification). Thus, the present method of gene mutation comprising irradiating sperm in presence of a psoralen derivative with UV light has surprising advantages when compared to methods suggested by the combined teachings of Chakrabarti et al., Grunwald et al. #1 and Grunwald et al. #2.

Thomas, et al, fails to overcome the limitations of the combined teachings of Chakrabarti et al., Grunwald et al. #1 and Grunwald et al. #2.

As the reference is understood, Thomas, et al., recites that irradiation of 4'-hydroxymethyl-4,5',8-trimethylpsoralen with near UV-light can induce **point mutation** in DNA found in human cell extracts. That is, Thomas recites that damage dependent replication errors T·A→C·G transitions, transversions at C·G base pairs, and deletions of single A·T base pairs are observed when DNA is exposed to 4'-hydroxymethyl-4,5',8-trimethylpsoralen and near UV-light. Thomas neither teaches nor suggests that small deletions in a vertebrate genome caused by induced by psoralen derivative and UV irradiation.

Moreover, one skilled in the art would not have been motivated by the Thomas teachings to select psoralen derivatives as a mutagen suitable for mutagenesis of mertebrate sperm in view of the variety of known mutagens available in the art.

Accordingly, the present invention would not be obvious to anyone skilled in the art from any of Chalrabarti et al., Grunwald et al. #1 or Grunwald et al. #2 taken with Thomas et al. Applicants request withdrawal of the rejection and reconsideration of the claims.

Reconsideration and withdrawal of the rejection of the noted claims are thus requested.

It is believed the application is in condition for immediate allowance, which action is earnestly solicited.

Respectfully submitted,

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